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# Opposing effects of behavioural activity and light on neurons of the suprachiasmatic nucleus

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## Abstract

The mammalian circadian pacemaker is located in the suprachiasmatic nuclei. It can be shifted in phase by photic cues and by the behavioural activity of the animal. When presented together, light and behavioural activity attenuate each others' phase-shifting effect. Still unclear is how behavioural activity affects the suprachiasmatic nuclei and how it interacts with photic information. Previously, we reported the occurrence of behaviourally induced suppressions of neuronal activity. The present study investigates the characteristics of these suppressions as a function of circadian time and, additionally, in the presence of photic cues. We performed long-term multiunit activity recordings of neurons in freely moving rats and found that these suppressions of neuronal firing in the suprachiasmatic nucleus occurred at every phase of the circadian cycle. The magnitude of the suppressions showed a circadian variation, with larger suppressions during subjective day. When a light pulse was applied during a suppression, light and activity appeared to oppose each others' effects within the recorded population of neurons. The resulting discharge level appeared to be the sum of both responses. The opposing effects of light and activity were also found in single unit recordings, indicating that photic and behavioural stimuli interact at the level of a single neuron.

## Introduction

The suprachiasmatic nuclei (SCN) of the hypothalamus contain the major circadian pacemaker in mammals. This pacemaker controls circadian rhythmicities in physiological functions, hormonal rhythms and behavioural activities such as the sleep/wake cycle (Meijer & Rietveld, 1989). Light is the most important external cue that entrains the circadian pacemaker. Illuminance information reaches the SCN via the retina and several ganglion cell projections (Moore & Card, 1990; Moga & Moore, 1997). Of these, the retino-hypothalamic tract is required for photic entrainment (Moore & Lenn, 1972; Johnson *et al*., 1988). Other photic afferents arise from the intergeniculate leaflet (Harrington *et al*., 1987), pretectum (Mikkelsen & Vrang, 1994) and raphe (Foote *et al*., 1978). Inside the SCN, the terminal fields of these projections overlap considerably with that of the retino-hypothalamic tract (Moore & Card, 1990; Moga & Moore, 1997).

The light responsiveness in discharge patterns of suprachiasmatic neurons has been measured using extracellular recording techniques *in vivo* (Groos & Mason, 1978; Inouye, 1984; Meijer *et al*., 1986, 1996; 1998). Most light-responsive SCN neurons react to light with an increment in firing frequency. Whilst phase shifts are induced only at night, light-induced changes in firing are present throughout the circadian cycle. Responses to light are maximal during subjective night, leading to enhanced intracellular responsiveness at this phase of the circadian cycle. The resulting elevation of intracellular messengers changes the expression of the transcript *mPer1*, which is the critical step for the generation of a light-induced phase shift (Akiyama *et al*., 1999).

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In the last decade, it has become evident that light is not the only phase-resetting stimulus. Among others, a number of neurotransmitters and increased behavioural activity induce phase shifts primarily during the day (Reebs & Mrosovsky, 1989). These behaviourally related stimuli appear to interact with photic entrainment. For instance, increased behavioural activity has the ability to inhibit light-induced phase shifts (Ralph & Mrosovsky, 1992; Mistlberger & Antle, 1998). Additionally, light pulses can inhibit the phase-shifting effects of behavioural activity (Mrosovsky, 1991; Joy & Turek, 1992; Mead *et al*., 1992; Biello & Mrosovsky, 1995). It is not yet clear at what level the interaction between photic and behavioural stimuli takes place.

Previously, transient suppressions in multiunit activity (MUA) have been shown in the SCN of freely moving rats and hamsters (Meijer *et al*., 1997; Yamazaki *et al*., 1998). These suppressions were associated with behavioural activity of the animal and lasted for about half an hour after the animal's activity had ended (Meijer *et al*., 1997). These neuronal suppressions reflect direct influence of the animal's activity on neuronal activity in the SCN and have been ascribed to the activation of specific afferents (Meijer *et al*., 1997; Yamazaki *et al*., 1998). We have now obtained a quantitative description of the behaviourally induced suppressions as a function of circadian time. Additionally, we observed the interaction between light responses and behaviourally induced suppressions at different phases of the circadian cycle.

## Materials and methods

Male Wistar rats were obtained from Harlan Zeist at the age of 9 weeks and housed in separate cages under a 12 : 12 h light : dark cycle. Procedures were as previously described (Meijer *et al*., 1998).

The animals were implanted with tripolar electrodes (stainless steel, diameter 0.125 mm, Plastics One, Roanoke, VA, USA). One electrode served as reference electrode and was placed in the cortex after removal of 0.5 cm of insulation at the outer end of the electrode. The other two electrodes were aimed at the suprachiasmatic nucleus with a distance of 0.4 mm between them. After surgery the animals were allowed to recover for at least 3 days before being transferred to the recording room, where the implant was connected to a counter-balanced swivel system. During the recording period animals could move freely and had free access to water and food pellets. Drinking activity was monitored and recorded by a separate computer system to determine circadian time. All efforts were to reduce the number of animals used. Animals were anaesthetized using a combination of Hypnorm (Janssen, Belgium) and Dormicum (Roche, The Netherlands). All experiments were approved by Dier-experimentele commissie.

The signal from the recording electrode was amplified, bandwidth-filtered and fed into spike triggers. One spike trigger was set to count multiple units from the signal. With a second spike trigger an attempt was made to isolate single neurons on the basis of spike amplitude. This was only possible when one of the neurons produced spikes of a large and distinctive amplitude compared to other units in the signal. During the recording session, uniformity of the spike waveform was checked daily on the oscilloscope. A third spike trigger was set above the largest spike amplitude. This spike trigger was used to detect electrical artifacts occurring during movements of the animal, such as locomotor activity, or during sniffing, drinking or chewing behaviour. Every 10 s the number of action potentials was read out by a computer, the counts were displayed on a screen for on-line monitoring and were stored for off-line analysis. Each bin in which movement-related artifacts were detected was excluded from the analysis.

We found that some, but not all, of these movement-related artifacts were followed by suppressions of neuronal activity in the SCN. An infrared camera was added to the recording setup and the behaviour of two animals that showed suppressions was observed in more detail. Video images were stored on tape for off-line comparison with the neuronal activity. The behaviour of these two animals was scored, and the following behavioural states were discriminated: (i) sniffing, (ii) eating, (iii) drinking, (iv) grooming, (v) head scratching, (vi) body scratching, (vii) shaking, (viii) lying down, (ix) changing body position, (x) digging, (xi) standing on hind legs and (xii) walking.

Within the recording room a continuous dark condition was maintained. A computer-controlled system provided the possibility to apply light stimuli of five different intensities (0.15–140 lux), which are within the working range of SCN neurons. Behavioural activity of the animals induced a long-term neuronal suppression lasting for about half an hour after the termination of the activity. Light pulses presented within this period were used to study the combined effects of behavioural activity and light at the neuronal discharge level.

### *Data analysis*

The off-line stored data were visually inspected for the occurrence of suppressions in multiunit activity. In 29 animals implantations were succesful, and in five out of 29 cases clear suppressions were observed. Of all recorded suppressions, we analysed only those that were nearly free from artifacts so that we had rather complete data sets. Because few suppressions were seen per 24 h of recording, a long period of recording was required for quantitative analysis. From one animal that showed supressions, a stable electrophysiological signal with an exceptional recording length of > 100 days was obtained.

The resulting data set provided sufficient suppressions to allow quantitative assessment of their attributes. The quantitative analysis was based on the observation that the suppressions recovered to baseline levels with an exponential-like function. The data sets were fitted by the least-squares method using Equation 1, where $t$ is time and $\tau$ the decay time constant. After the initial fit, outliers (deviating from the model by at least 2 × SEM) were removed and the fit was repeated. The resulting parameters were tested for circadian variation using Hotelling's Trace (Hotelling, 1931).

$$\widehat{\mathrm{MUA}} = \mathrm{offset} + \mathrm{gain} \cdot (1 - \mathrm{e}^{-t/\tau}) \quad (1)$$

To analyse the interaction between the effects of behaviourally induced suppressions and the applied light pulses, the MUA response to every light pulse and also the MUA prior to the light pulse was scored. When a suppression had occurred during a light pulse, we additionally measured the MUA preceding the suppression. The interactions between light and suppressions were assessed by two-way ANOVA. Results were considered statistically significant when the probability level was 0.05 or lower ($P < 0.05$). Circadian rhythmicity of the baseline discharge patterns were verified by Lomb periodogram analysis (Meijer *et al*., 1998).

## Results

We have obtained stable MUA recordings of a total length of 103 days from one animal with behaviourally induced suppressions (Fig. 1, upper panel). Whilst only a small fraction of all behavioural activities resulted in suppressions, every suppression was preceded by behavioural activity (Fig. 1, lower panel). This raises the question of whether a specific behavioural state elicited the neuronal suppressions. Our recording technique did not allow discrimination between different types of behavioural activity, because all the movements of the animal were detected by the electronic setup. Additional infrared video recordings in two animals revealed that behaviours such as feeding, drinking, sniffing, grooming and scratching did not by themselves evoke a neuronal suppression. Instead, most suppressions were accompanied by locomotor activity.

A total number of 55 suppressions were fitted, of which 31 occurred during subjective day and 24 during subjective night. Despite quite some variability in the baseline MUA (Meijer *et al*., 1997) the slow time course of the suppressions allowed good estimation of the model parameters (variance explained ± SEM, 79 ± 1%).

The minimal firing rate at the beginning of the suppressions (offset) showed large variations between consecutive suppressions (Fig. 1). The speed of the recovery to baseline levels and the offset did not vary with circadian time (Figs 2 and 3). The amplitude of the suppressions was larger during subjective day compared to subjective night (2-way ANOVA, Hotelling's Trace, $P < 0.001$; amplitude, $P < 0.001$; Figs 2 and 3). The MUA level at the end of the suppressions (final value) was calculated as the sum of the mean offset and the amplitude (gain) of the suppressions (Fig. 3). The final value exhibited a circadian rhythm. This rhythm is caused by the rhythm in baseline MUA in the SCN.

We determined the characteristics of the suppressions in four other animals. In these animals, time constants were 11 ± 2 min, range 6–23 min, $n = 13$. The minimal firing level that was reached during a suppression (offset) varied among the different animals and ranged from 40 to 95% of the baseline activity. The mean amplitude of the suppressions was 68 ± 5 Hz (range: 44–72 Hz; $n = 13$). We conclude that the major characteristics that we described quantitatively for one animal, namely (i) the suppression occurred at every phase of

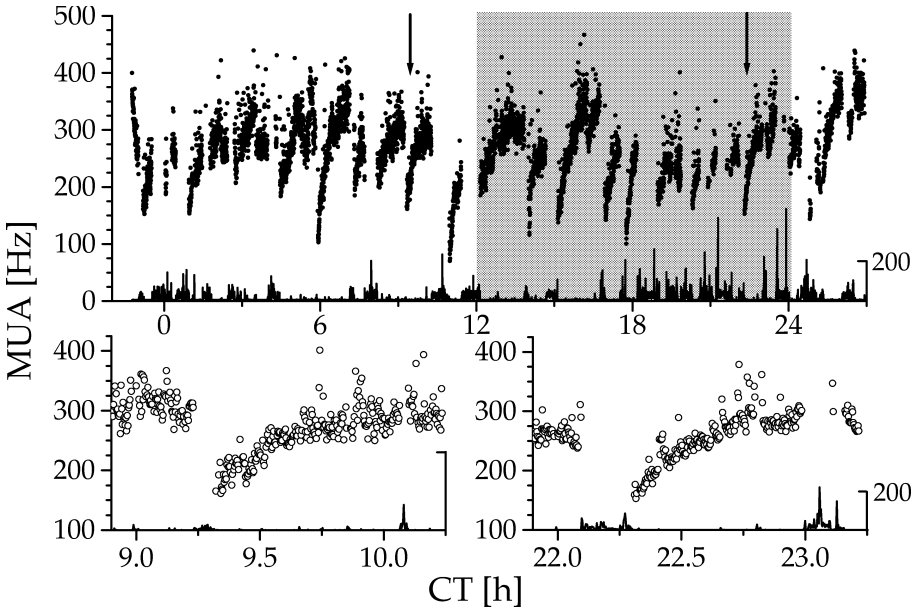


FIG. 1. Suppressions in MUA recorded in continuous dark (DD). In the upper figure, MUA has been plotted against circadian time (CT), which has been determined by assessing the drinking activity of the animal. Subjective night is indicated by shading. The electronically detected behavioural activity is shown along the horizontal axis (see Materials and methods). Black dots represent MUA samples of 10 s duration. Arrows denote suppressions that are enlarged in the lower panels, where MUA is plotted with open circles. The scale bars to the right of each panel refer to behavioural activity and represent 200 Hz.

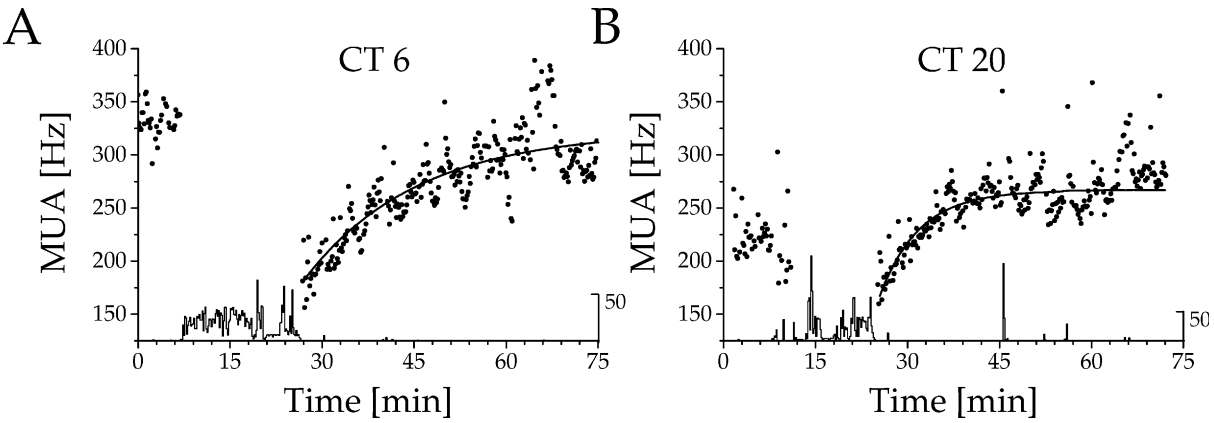


FIG. 2. Neuronal suppressions and fits. Two examples of suppressions are shown, one obtained during subjective day (A) and one during subjective night (B). The circadian times are given above the traces. MUA (in black dots) is plotted together with the resulting fit (solid line). Behavioural activity is depicted on the horizontal axis.

the circadian cycle and (ii) the mean offset of the suppressions was constant, were also found in the four other animals (Fig. 4).

The electrode placements were verified histologically. Three electrodes were located in the ventral SCN, one electrode was located on the anterior border of the SCN and the fifth electrode was located in the optic chiasm, immediately ventral to the SCN. None of the electrodes were found in the posterior areas of the SCN, or in the dorsal aspects. Comparison of these locations to electrode placements in animals that did not show suppressions ($n$ = 10) revealed a rather large overlap in the recording area. Thus, the placement of the electrodes did not relate to the occurrence of the suppressions.

To determine whether the cellular effects of behavioural activity interacted with the responsiveness to light, light pulses were applied during suppressions. By themselves, light pulses induced an increase in neuronal activity throughout the circadian cycle. We found that light pulses opposed activity-induced suppressions (Fig. 5).

To quantify the interaction between the effects of behavioural activity and retinal input, the magnitude of light responses was measured in two ways (Fig. 6). We measured the magnitude of light responses during a suppression, relative to the discharge level immediately preceding the light pulse. The magnitude of light responses during a suppression was not different from that of control light responses (light response during suppression, 166 ± 10 Hz; control light response, 180 ± 5 Hz; 2-way ANOVA, not significant). This means that the magnitude of the light response itself was not changed by a suppression. We also measured the obtained firing rate during a light pulse relative to the firing rate before the suppression. We found that the obtained change in discharge rate induced by a light pulse and a suppression together was smaller than the change in discharge rate induced by light alone (light response during suppression, 112 ± 10 Hz; control light response, 180 ± 5 Hz; 2-way ANOVA, $P < 0.001$, Fig. 6). It follows that the combined effect of light and behavioural activity is predictable from a simple addition of their separate effects.

We were able to record single unit activity for five consecutive cycles and observed: (i) suppressions of neuronal firing following

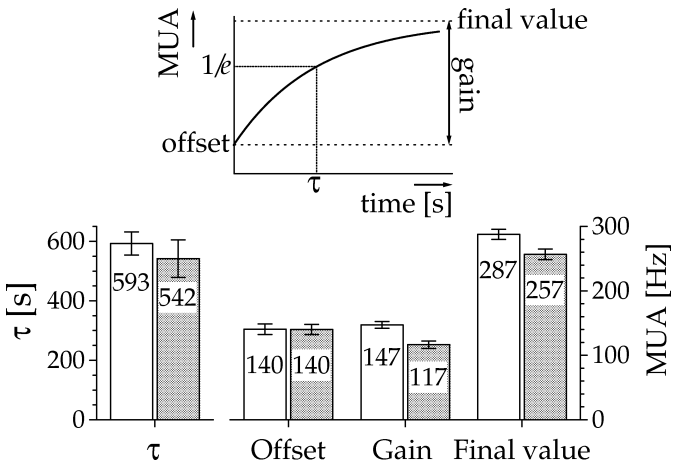


FIG. 3. Fitted parameters during subjective day (white) and night (grey). In the upper panel the exponential function (Equation 1) by which the suppressions were fitted is shown. The meaning of several terms has been indicated in the plot. In the lower part of the figure the values of parameters during both subjective day and subjective night are shown with the SEM.

behavioural activity of the animal; (Fig. 7A) (ii) excitatory responses to light pulses (Fig. 7B) and (iii) combined effects of both suppressions and light responses (Fig. 7C). The time constant of the suppression fell within the range of those observed in MUA recordings. However, single unit activity was completely blocked, whereas MUA dropped to a minimal firing level.

## Discussion

In this study, behaviourally induced suppressions in firing rate of suprachiasmatic neurons have been characterized in five animals. During the suppressions the firing rate decreased to 40–95% of the baseline activity, depending on the animal. The neuronal firing recovered with a time constant of ≈10 min. The suppressions occurred at every phase of the circadian cycle. The recovery of the suppressions started from a minimal level of firing rate which did not differ between subjective day and night. A circadian rhythm in the amplitude of the suppressions was observed, with larger suppressions during the day.

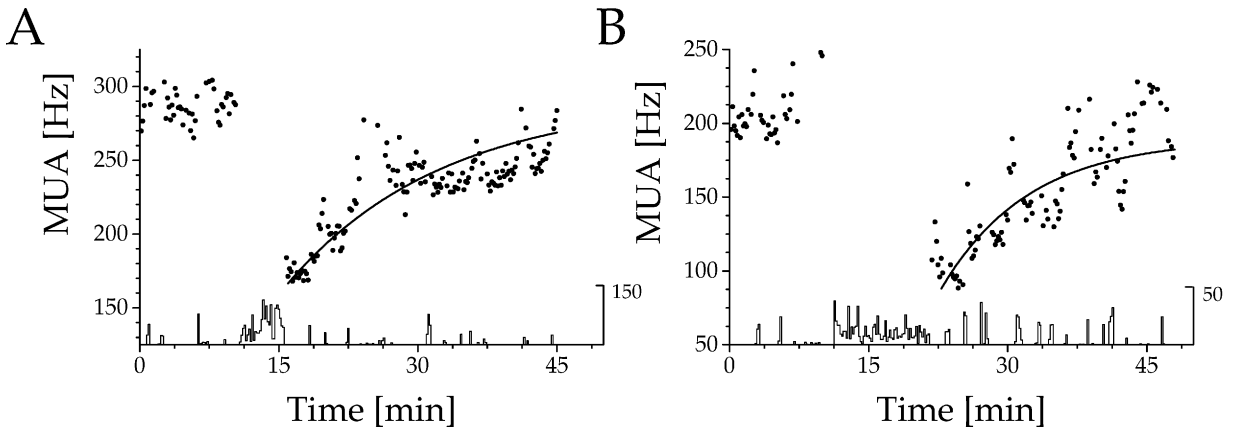


FIG. 4. Behaviourally-induced suppressions in MUA in two other animals. Two suppressions are shown, together with the fits. (A) Suppression of MUA at CT 4 (8 h before activity onset). Behavioural activity is given on the horizontal axis. Dots represent MUA per 10 s. (B) Suppression of MUA in another animal at CT 7. Plotting conventions are as in A.

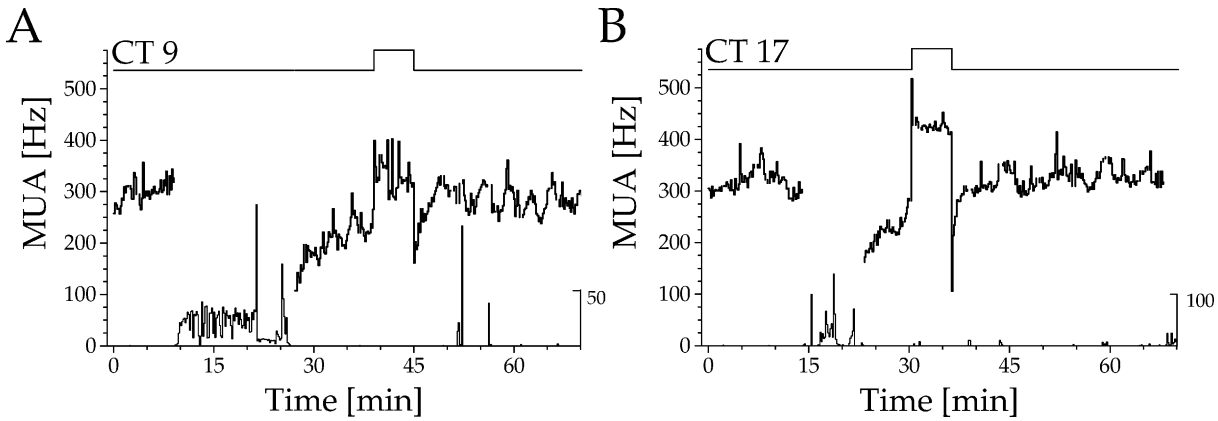


FIG. 5. Light responses and behaviourally induced suppressions in neuronal activity. Two examples of responses to light pulses that are presented to the animal during suppressions of MUA are shown. The timing of the light pulses is indicated above the figure and the behavioural activity is plotted at the bottom of the figure. The ability of light to bring the firing rate above the basal firing frequency depended on the circadian time of light exposure for this particular light intensity (0.15 lux). (A) During subjective day, light responses were small, whereas (B) during subjective night, light responses were larger, raising the firing rate above baseline levels despite the presence of a neuronal suppression.

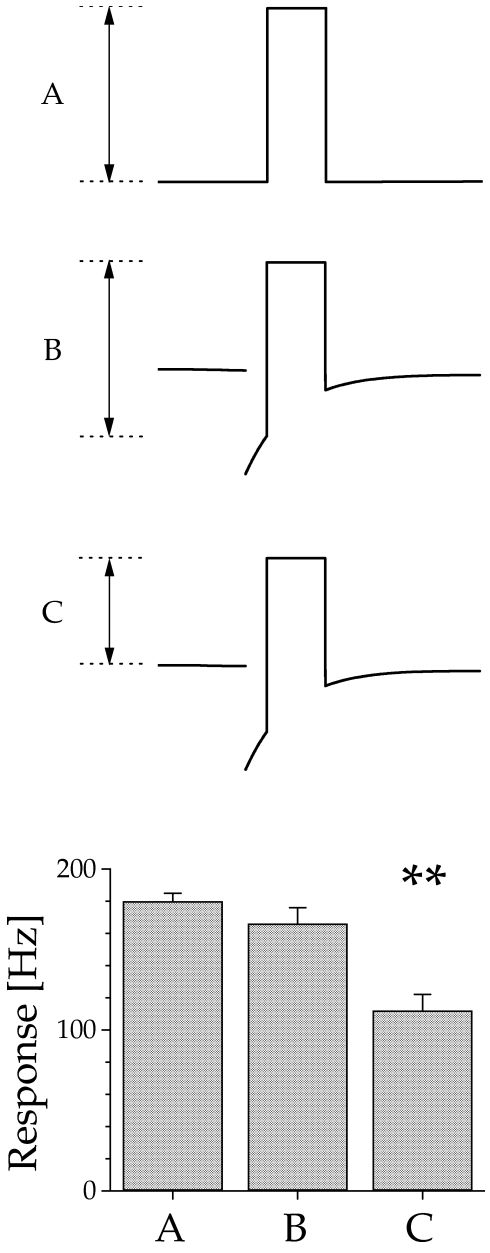


FIG. 6. Quantification of the interaction between light responses and neuronal suppressions. (A–C) The individual drawings show how the interaction was measured. (A) Control light responses were measured relative to the baseline discharge rate. (B) The measurement of a light response during a suppression. The reference value was the discharge rate immediately prior to the light pulse. (C) The measurement of a light response during a suppression. The reference value was the baseline discharge rate that was recorded immediately before the suppression. The bar plots A–C show the results of these measurements. The values represent the average responses to light pulses with SEMs. $^{**}P < 0.001$ vs. the control light response A. Comparison of A and B reveals that the magnitude of the light response has not changed as a consequence of the suppression. It is concluded that the combination of the two stimuli results in a discharge rate that can be calculated from an addition of the separate responses.

### *Quantification of neuronal suppressions*

Quantitative analysis of MUA signals is hampered by variations in electrode placement among animals. As the suprachiasmatic neurons form a heterogenous population, with both anatomically and electrophysiologically defined differences (Moga & Moore, 1997; Schaap *et al*., 1999), characteristics of a certain subpopulation may dominate in one MUA recording and may be completely absent or only partially detectable in the next. This heterogeneity probably also underlies the low occurrence of animals with suppressions (5 out of 29). The suppressions may only be visible when neurons with a certain property (such as afferent connections from the intergeniculate leaflet) are represented sufficiently in the measured population. For instance, 27% of visual SCN neurons are suppressed by light and 73% are light-activated (Meijer *et al*., 1986). Nevertheless, light-suppressed neurons are hardly ever detectable in multiunit recordings (< 10%) and are probably overruled by light-activated responses.

The proportion of recordings in which we observed activity-induced suppressions is much lower than the proportion of recordings in which light responses were obtained, which may indicate that the visual input is stronger than the behavioural input. Noteworthy, in the hamster, behaviourally-induced suppressions are commonly observed (Yamazaki *et al*., 1998). This may relate to the stronger phase-shifting effect of behavioural activity in the hamster, but this is not yet clear**.**

Another complication is the low number of spontaneous suppressions per circadian cycle. A large number of circadian days is therefore needed to obtain sufficient data. In this paper we have presented the case of one animal from which > 100 days of MUA signals have been obtained, all originating from the same population of neurons. This data set provided the opportunity to characterize the suppressions with respect to their shape and magnitude, their circadian variation and their possible interactions with photic input.

On the basis of our quantitative results the MUA suppression have been reconstructed mathematically to provide a simple model. In this reconstruction the stability of the mean offset and time constant $\tau$ are clear, as well as the circadian rhythm in the amplitude of the suppressions (Fig. 8A). We determined the characteristics of suppressions in four other animals. The characteristics appeared to be equal to the model, with the exception of the mean offset, which varied among the animals. We ascribe this to different subpopulations of SCN neurons that contribute to a particular recording. We conclude that suppressions in MUA of other animals were consistent with this model.

Light pulses were applied to study the combined effects of light and activity. Application of light leads to an increment of MUA within the SCN in most animals (Inouye, 1984; Meijer *et al*., 1996, 1998). The separate as well as the combined effects of the two inputs were observed at all phases of the circadian cycle. The results have been integrated into our model, showing that light responses are highest during subjective night whilst suppressions have a minimum amplitude during this period (Fig. 8B–C). These results show that both types of stimuli are able to reduce each others' effects.

Light also affects the behavioural state of the animal directly; this phenomenon is called 'masking'. Nocturnal animals generally cease their activities when lights are on, regardless of the state of the circadian pacemaker. In our study, we analysed the effects of light on SCN discharge on moments that the animal was not active so that an additional masking effect of light on behavioural activity could be excluded**.**

### *Neuronal suppressions and phase shifts*

The neuronal suppressions may be causally related to phase shifts that are induced during the day. Examples of such stimuli are dark pulses, novel wheel exposure, cage cleaning and conditioned stimuli (Mrosovsky, 1988; Van Reeth & Turek, 1989; Amir & Stewart, 1996; Bobrzynska & Mrosovsky, 1998). Also, activity increments that may occur as a side-effect of a pharmacological injection can

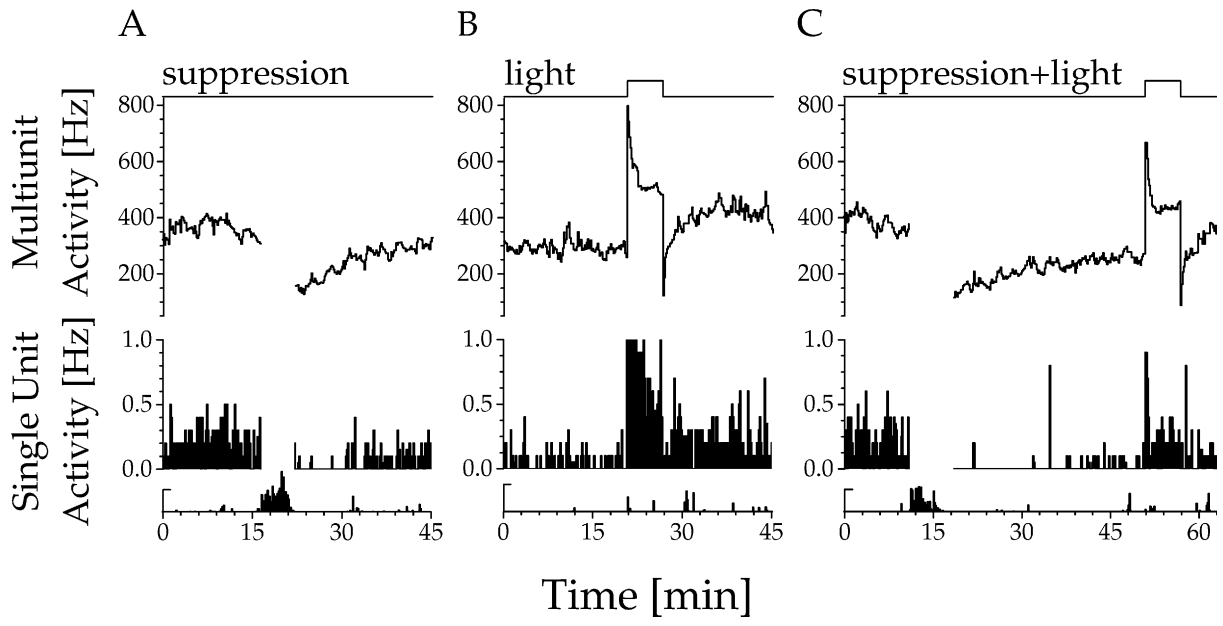


FIG. 7. Behaviourally-induced suppressions and light responses in single unit recordings. Multiunit activity is given in the upper traces per 10 s; single unit activity is plotted underneath. Behavioural activity is indicated on the horizontal axes below the graphs, the scale bars denoting 200 Hz. Plotting conventions are as in Fig. 5. (A) Example of a behaviourally-induced suppression in multi- and single unit activity. (B) Example of a light response (140 lux, 6 min) in multi- and single unit activity. (C) Example of a suppression and a light response (140 lux, 6 min) in multi- and single unit activity. The examples are taken from circadian times 9, 9 and 10, respectively. The characteristics of the single unit response are similar to the multiunit response, the only difference being the magnitude of the suppression as the discharge of the single unit reached the zero level for several minutes.

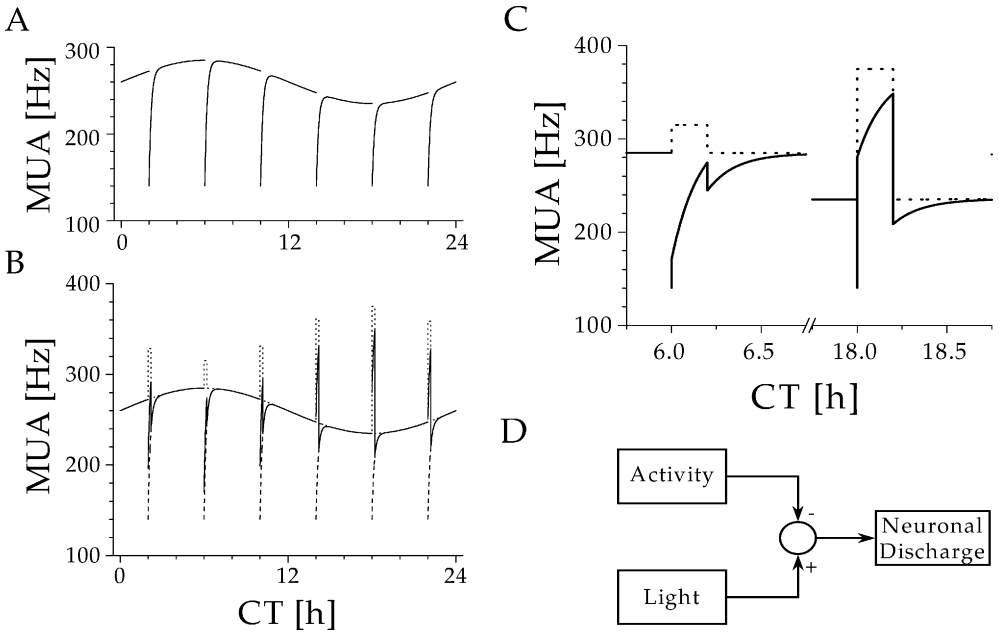


FIG. 8. Reconstruction of baseline MUA, suppressions and responses to light pulses as a function of circadian time. (A) MUA is represented by a sine wave form. The suppressions have been plotted according to the parameters obtained in this paper, and show a stable mean offset and time constant throughout the circadian cycle. (B) Using the results from the two-way ANOVA analysis (see Results), the responses to light (0.15 lux) have been added to the model and are plotted with dotted lines. For comparison, the data from A are replotted with a dashed line. The response to light application during a suppression is plotted with a solid line. (C) For clarity, the combined response (solid line) as well as the response to control light pulses (dotted line) are shown on an enlarged time-scale, for mid-subjective day and mid-subjective night. (D) The interaction is also represented in a flow diagram.

induce phase shifts (Turek & Van Reeth, 1988; Wee & Turek, 1989; Maywood *et al*., 1997). It is unknown what the actual stimulus is that causes these shifts. Although the occurrence of activity is a reasonable predictor for the occurrence of phase shifts, some animals do not show shifts despite considerable amounts of running-wheel activity. It has been suggested that it is motivated activity more than

induced activity that induces phase shifts (Mistlberger, 1991; Mrosovsky, 1996). Similar uncertainty exists with respect to activity-induced suppressions of neuronal SCN activity. Yamazaki *et al*. (1998) found suppressions in response to running wheel activity of the hamster. In our study, where cages were not equipped with a running wheel, increased locomotor activty was the best predictor for the occurance of neuronal suppressions. However, there were instances when the rats appeared active in their cage, but no suppressions were observed.

Both serotonin and neuropeptide Y (NPY) may play a role in activity-induced phase shifts (Edgar *et al*., 1993; Biello *et al*., 1994; Janik & Mrosovsky, 1994; Bobrzynska *et al*., 1996; Marchant *et al*., 1997). These transmitters are found in the projections of the raphe and the intergeniculate leaflet to the SCN (Moga & Moore, 1997). Although NPY elicits both inhibitory and excitatory responses, the suppressive effect of NPY is probably dominant (Schmahl & Bohmer, 1997; Gribkoff *et al*., 1998). The inhibitory action of serotonin (Meijer & Groos, 1988) and NPY may underly the suppressing effect of behavioural activity on SCN discharge.

It is unclear whether these suppressions in discharge are involved in phase shifting. Suppressions in firing rate generally result from a hyperpolarization of membrane potential, moving it away from firing threshold. There are strong indications that NPY-induced hyperpolarizations of membrane potential are not required for phase shifting by NPY (Gribkoff *et al*., 1998; Hall *et al*., 1999). However, extrapolation of this study to the role of hyperpolarizations in phase shifting in general is difficult, as hyperpolarizations can be driven by various neurotransmitters and channels, which lead to different intracellular processes. Therefore, the phase-shifting capacity of activity-induced suppressions remains to be clarified.

### *Interactions between neuronal suppressions and photic responses*

Photic entrainment relies on a phase-dependent responsiveness of the circadian pacemaker to light. Light pulses presented during the early night produce phase delays of the circadian pacemaker whereas during the end of the night they produce phase advances. Furthermore, light pulse presentation during the subjective day will not elicit phase shifts. *In vivo* multiunit recordings in rats have demonstrated that light pulses induce sustained increments in neuronal discharge in the SCN (Inouye, 1984; Meijer *et al*., 1996). The responses are triggered throughout the circadian cycle, the amplitude being dependent on circadian time and intensity (Meijer *et al*., 1998).

Our recordings revealed that behavioural activity has acute effects on SCN neuronal activity. This mechanism may play a modulating role in concert with light entrainment. These modulations were also observed in single unit activity, indicating that individual SCN neurons receive dual inputs and may integrate them (Fig. 8D).

This finding is consistent with anatomical data which show that the projection sites of the retino-hypothalamic tract, the geniculo-hypothalamic tract and afferents from the raphe all terminate in the ventral subdivision of the rat SCN (Moore & Card, 1990; Moga & Moore, 1997). The locations of the recording sites in this study all fall in this subdivision. The occurrence of interactions within single cells are an indication that photic input and behavioural activity interact at the level of the membrane potential by de- or hyperpolarizing mechanisms.

Several findings suggest an interaction between behavioural stimuli and photic cues at a functional level. Induced activity is capable of inhibiting phase shifts evoked by photic events (Ralph & Mrosovsky, 1992). Conversely, phase shifts evoked by behavioural activity are attenuated by light pulses (Mrosovsky, 1991; Joy & Turek, 1992; Mead *et al*., 1992; Biello & Mrosovsky, 1995). The opposing effects of light and activity on SCN neuronal firing may reflect the underlying cellular basis for these interactions and could be consistent with studies showing that NPY and glutamate affect each others' phase shifts *in vitro* (Biello *et al*., 1997).

### *Circadian rhythm in neuronal suppressions and photic responses*

The MUA recordings show that the relative amplitude of responses to light and behavioural activity change throughout the circadian cycle. Whilst neuronal suppressions are maximal during the day, the photic responses are maximal during subjective night (Fig. 8C). These data suggest that photic stimuli of high magnitude are needed during subjective day, if the light is to inhibit phase shifts induced by behavioural stimuli. Substantial confirmation of this notion is to be found in studies in which a combination of stimuli was used. To block activity-induced phase shifts during subjective day by photic cues, light pulses of saturating intensity have been applied (Mrosovsky, 1991; Joy & Turek, 1992; Mead *et al*., 1992; Biello & Mrosovsky, 1995; Penev *et al*., 1997), on average 350 lux for one hour. In experiments that investigate the ability of behavioural stimuli to inhibit light-induced phase shifts during subjective night, relatively short and dim light pulses have been used (Ralph & Mrosovsky, 1992; Rea *et al*., 1994; Pickard *et al*., 1996; Bradbury *et al*., 1997; Weber & Rea, 1997; Mistlberger & Antle, 1998; Amir & Stewart, 1999), on average 30 lux for 15 min. This differentiated use of light pulse magnitude confirms our findings regarding the relative potency of light and behavioural activity to antagonize each others' effect at the different phases of the circadian cycle.

We conclude that the relative ability of inputs to modify SCN neuronal activity exhibit a circadian rhythm and may determine the characteristics of resulting phase shifts. Because the combination of photic inputs with behavioural stimuli is an important factor in several contexts, such as shift work and sleep disorders in the elderly (Mrosovsky, 1988; Ralph & Mrosovsky, 1992; Hastings, 1997; Penev *et al*., 1997), a theoretical framework to predict and explain the combined results of different inputs is useful. Studies of SCN responses in neuronal discharge *in vivo* form an important contribution to our understanding of how the circadian pacemaker processes incoming information.

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## Abbreviations

MUA, multiunit activity; NPY, neuropeptide Y; SCN, suprachiasmatic nucleus.

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